Art rioscleros is, Thrc osis, And Vascular Biology:

Inflammatory and Thrombotic Modulators of Vascular Disease:

Monday Morning
Convention Center Room 109B
Abstracts 191 - 200

191

The Urokinase Receptor Interacts with the Extracellular Domain of the CD11b Subunit and Modulates Mac-1 (CD11b/CD18) Function

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The integrin Mac-1 and the glycolipid (GPI) anchored urokinase receptor are physically linked and reciprocally modulate each other's functions in monocytic cells. This study defines a region within CD11b with which uPAR interacts and thereby modifies Mac-1 function. Soluble human uPAR (suPAR) lacking the GPI anchor inhibited Mac-1-mediated fibrinogen (FGN) binding and turnover by monocytic THP-1 cells (IC_{50} =100 nM). Activation of Mac-1 with the stimulating mAb KIM 185 potentiated inhibition by suPAR (IC_{50} =0.1 nM), suggesting that an extracellular interaction between uPAR and Mac-1 may be functionally important. suPAR bound to soluble, purified and activated Mac-1 and this binding was inhibited by a peptide which binds to domain 2/3 of uPAR. In contrast, significantly less uPAR (< 80%) bound to soluble LFA-1 (CD11a/CD18), implying that the CD11b-subunit is required for the interaction between uPAR and Mac-1. The importance of CD11b was investigated by transfecting erythroleukemic K562 cells, which lack Mac-1, with the isolated CD11b-subunit. The specific, CD11b-dependent binding of FGN observed with these transfected cells was found to be regulatable by uPAR. To further define the domains within CD11b responsible for this interaction with uPAR, a series of chimeric human CD18 integrins paired with various fusions of CD11b and CD11c (p150,95) were co-transfected into CHO cells expressing human uPAR. The ability of uPAR to regulate Mac-1-dependent FGN binding and degradation was preserved in one of the five CD11b/CD11c CHO cell chimeras which retained the region spanning the I- and cation-binding domains of CD11b. Thus, the interaction between domain 2/3 of uPAR and a defined region within CD11b regulates Mac-1 function and provides a therapeutic approach to modulate inflammation.

192

Inhibition of Macrophage Homing to Atherosclerotic Plaques in ApoE Deficient Mice by Anti-α_4 Antibody

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Monocytes/macrophages play a central role in the development of atherosclerotic plaques. A better understanding of the mechanism of attachment of monocytes to activated endothelial cells may prove useful in developing strategies aimed at attenuating the development/progression of atherosclerosis. Here, we describe a novel *in vivo* model that directly demonstrates homing of macrophages to atherosclerotic plaques. Macrophages were loaded with fluorescent microspheres and injected intravenously into 40-week old Apolipoprotein E-deficient mice. After 48 hours, labeled macrophages were observed adhering to atherosclerotic plaques and also to organs of the reticuloendothelial system, namely the liver and spleen. The mean number of macrophages adherent to atherosclerotic plaques located in the proximal 1 mm of the aortic root just above the aortic valve was quantitated to be 140±16 macrophages (n=6). Pretreatment with a monoclonal antibody directed against the α-subunit of the $\alpha_4\beta1$ integrin reduced macrophage homing to the aortic root by 75% as compared with isotype-matched control (44±15 cells vs. 177±25 cells, p=0.0002)(n=10). The ability to reduce macrophage homing to the early sites of atherogenesis by blocking the α-subunit of the $\alpha4\beta1$ integrin and its counter-receptors may provide a means to attenuate the progression of atherosclerosis.

193

The Lack of a Leukocyte IL-8 Receptor Homologue Leads to Marked Inhibition of Atherosclerosis in LDL Receptor-Deficient Mice

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Leukocyte-mediated inflammation modulates atherogenesis and expression of the monocyte chemotactic C-C chemokine, JE/MCP-1, is believed to play a role. However, the C-X-C chemokines, IL-8 and GROα, which bind to common IL-8 receptors (IL-8R) and are best recognized as neutrophil chemotaxins, also can be expressed in atherosclerotic lesions and can mediate T-lymphocyte adhesion to endothelial cells. To understand the role of C-X-C chemokines in atherogenesis, we irradiated 6 week old, male LDL receptor-deficient (LDL-R-/-) mice to eliminate their endogenous bone marrow-derived cells. Half of the mice were repopulated with bone marrow cells from mice deficient in the homologue of human IL-8R's (LDL-R-/- → IL-8R-/-, n=11). To serve as controls, the other mice received bone marrow cells from wild-type mice (LDL-R-/- → W/T, n=11). RT-PCR analysis confirmed that LDL-R-/- → IL-8R-/- mice had no peripheral blood leukocyte IL-8R expression. Four weeks after transplantation, all mice were fed an atherogenic diet for 16 weeks to induce atherosclerosis. Upon sacrifice, the LDL-R-/- → IL-8R-/- mice exhibited splenomegaly and a lack of germinal centers in their spleen, which are known characteristics of the IL-8R-/- mice. They also weighed ~15% less than the LDL-R-/- → W/T mice. Plasma cholesterol increased dramatically in both groups upon feeding the atherogenic diet, with the levels ~30% higher in the LDL-R-/- → W/T mice compared to LDL-R-/- → IL-8R-/- mice. Quantitation of serial sections of Oil Red O-stained aortic valve lesion areas revealed that the lesions were reduced 2-3 fold in the LDL-R-/- → IL-8R-/- mice compared to the LDL-R-/- → W/T mice. Our findings suggest that IL-8R expression on bone marrow-derived cells plays an important role in atherogenesis in LDL-R-/- mice.

194

The V-Domain of Receptor for Advanced Glycation Endproducts (RAGE) Mediates Binding of AGEs: A Novel Target for Therapy of Diabetic Complications

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Nonenzymatic glycation/oxidation of proteins, a critical consequence of hyperglycemia, results in the irreversible formation of Advanced Glycation Endproducts (AGEs). AGEs, which accumulate in plasma/tissues, impart their pathogenic effects via interaction with cellular receptors, the best-characterized is Receptor for AGE (RAGE). AGE-RAGE interaction results in vascular/inflammatory cell dysfunction, which is inhibited in the presence of soluble or sRAGE, the extracellular (EC) portion, composed of one V-type domain followed by two C-type Ig domains. In vivo, administration of sRAGE blocks vascular hyperpermeability and hyperfibrinogenemia in diabetic rodents. sRAGE suppresses accelerated atherosclerosis in diabetic Apo E null mice and improves wound healing in insulin-resistant db+/db+ mice. To delineate which portion of sRAGE mediates these effects, we developed anti-peptide antibodies against regions in the three EC domains. While antibodies against V-domain peptides completely inhibited binding of ^{125}I-sRAGE to immobilized AGE, antibodies against C1 or C2 peptides had no effect. Soluble V-domain blocked binding of radiolabelled sRAGE to AGE, soluble C1 and C2 domain had no effect. ^{125}I-soluble V-domain bound immobilized AGE with K_d =68± 6.9 nM, similar to that of intact sRAGE. Linear peptides were then prepared composed of either 1-30 or 31-60 amino acid regions in the V domain. 1-30 inhibited the binding of ^{125}I-sRAGE (100nM) >90% to AGE, even at 10-fold molar excess concentration. Peptide 31-60 was without effect. These data indicate that the critical interaction site of AGEs with RAGE lies in the V-domain, likely within its first 30 amino acids. This region may be a novel target in the design of agents to prevent/interrupt diabetic complications.

195

Anti-PDGF Beta-Receptor Antibody Inhibits Neointima Formation in Primates

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The proliferation of smooth muscle cells (SMC) and production of extracellular matrix contribute to vascular lesion development in injured arteries. Platelet derived growth factor (PDGF) is a potent chemotactic agent and mitogen for SMC that may contribute to this process. Therefore, we assessed the effects of blocking the PDGF beta-receptor in baboon models of vascular injury. Ten baboons underwent balloon angioplasty of one femoral artery and had stents (Palmaz-Schatz) placed in their carotid arteries. Five animals were treated with an anti-PDGF beta-receptor monoclonal antibody (1 mg/kg) for 6 days. In vitro, this antibody was shown to block PDGF ligand binding, PDGF-induced SMC mitogenesis, and PDGF receptor autophosphorylation. The remaining 5 animals served as controls. All tissues were harvested at 30 days. The femoral arteries were embedded in paraffin, while the stents were embedded in methacrylate. Morphometric analysis was performed to measure neointimal area. Neointima formation after femoral balloon angioplasty was reduced 38% by antibody treatment ($p<0.05$ vs. the controls). Similarly, the size of neointimal lesions in stented vessel segments was reduced by 26% ($p<0.05$). Scanning electron microscopy revealed that the stents were covered with a consistent layer of endothelial cells. This study documents that one week of therapy with an anti-PDGF receptor antibody can significantly reduce lesion size at one month, suggesting an important role for PDGF in early proliferative events. Further, the antibody reduced lesion size following two types of vascular injury: simple balloon angioplasty and placement of a chronic stent. Overall, these studies in primates suggest that targeting the PDGF pathway may be a promising strategy for limiting restenosis after mechanical vascular injury.

196

Use of a Transfected Cell Line to Identify a Small Molecule, Non-peptide Macrophage Scavenger Receptor Antagonist

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Macrophage scavenger receptor (MSR) antagonists may prevent foam cell formation and the initiation of atherosclerosis, since a recent report found that MSR/apoE double-knockout mice had 60% smaller lesions than apoE single-KO littermates. We constructed a screening cell line, examined chemical libraries, and found putative small molecule MSR antagonists. Full length clones of MSR-I and MSR-II receptors were isolated from a human placental library, subcloned into the expression vector pCDN, and transfected into HEK 293 cells as stable cell lines. A 96-well plate screening assay was optimized for 4hr of uptake at 2μg/ml of DiI-AcLDL. Polyinosine competed with an IC_{50} of 1μg/ml; dextran sulfate with an IC_{50} of 1.7μg/ml; fucoidin with an IC_{50} of 11μg/ml; LDL with an IC_{50} of ≈100μg/ml; and the compound (E)-methyl 4-chloro-α-[4-(4-chlorophenyl)-1,5-dihydro-3-hydroxy-5-oxo-1-(2-thiazolyl)-2*H*-pyrrol-2-ylidene]benzeneacetate with an IC_{50} of 6μg/ml (17μM). With ^{125}I-AcLDL as ligand for 293 cells in 24-well dishes, binding/uptake at 37C for 5 hr was saturable with an apparent k_d of 11μg/ml and a B_{max} of 6525ng/5hr/mg protein. ^{125}I-AcldI degradation yielded a k_d of 5μg/ml with a B_{max} of 2680 ng/5hr/mg protein. Poly-I competed both ^{125}I-AcLDL binding and degradation with an IC_{50} of 1.5μg/ml; dextran sulfate with an IC_{50} of 2μg/ml; and the small molecule with an IC_{50} of 38μM.

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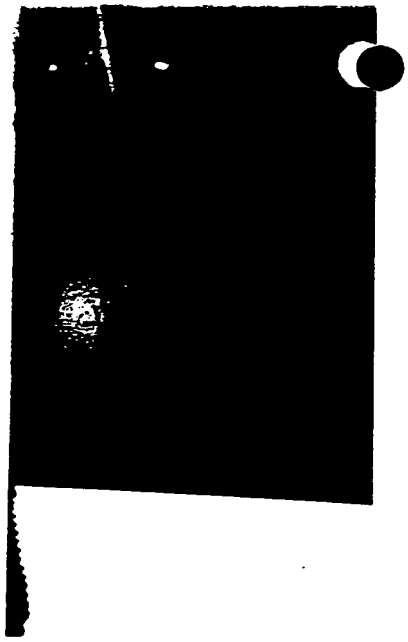

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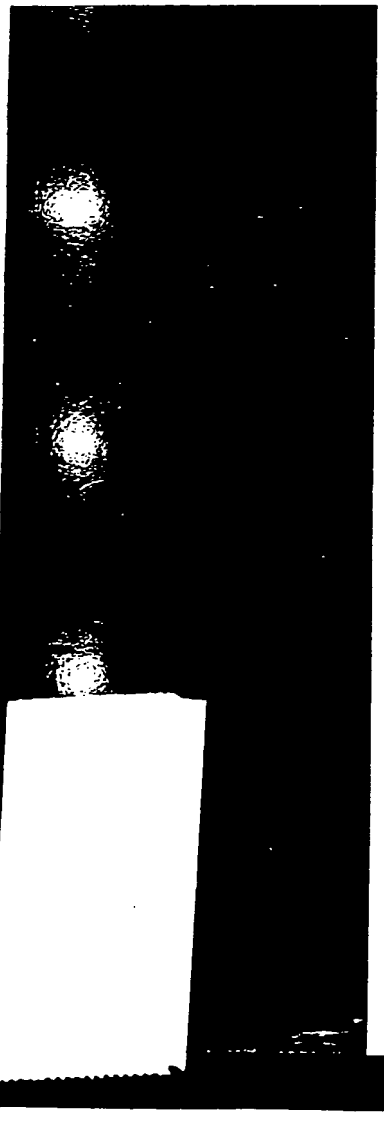

Named and Invited Lectures

Lewis A. Conner Memorial Lecture • Helen B. Taussig Memorial Lecture • Paul Dudley White International Lecture • Thomas W. Smith Memorial Lecture • William W. L. Glenn Lecture • Ancel Keys Lecture • Laennec Society Invited Lecture • Charles T. Dotter Memorial Lecture • Dickinson W. Richards Memorial Lecture • George E. Brown Memorial Lecture • Lewis K. Dahl Memorial Lecture • George Lyman Duff Memorial Lecture • Sol Sherry Lecture in Thrombosis

Young Investigator Award/Prize Abstracts

The Council on Cardiovascular Nursing New Investigator Awards • Samuel A. Levine Young Investigator Awards • Melvin L. Marcus Young Investigator Awards • Cournand and Comroe Young Investigator Prizes • Louis N. and Arnold M. Katz Basic Science Research Prizes • Vivien Thomas Young Investigator Awards • Melvin Judkins Young Clinical Investigator Award • Irvine H. Page Research Awards • Elizabeth Barrett-Connor Research Awards • Young Investigator Awards in Thrombosis

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